FIG. 1

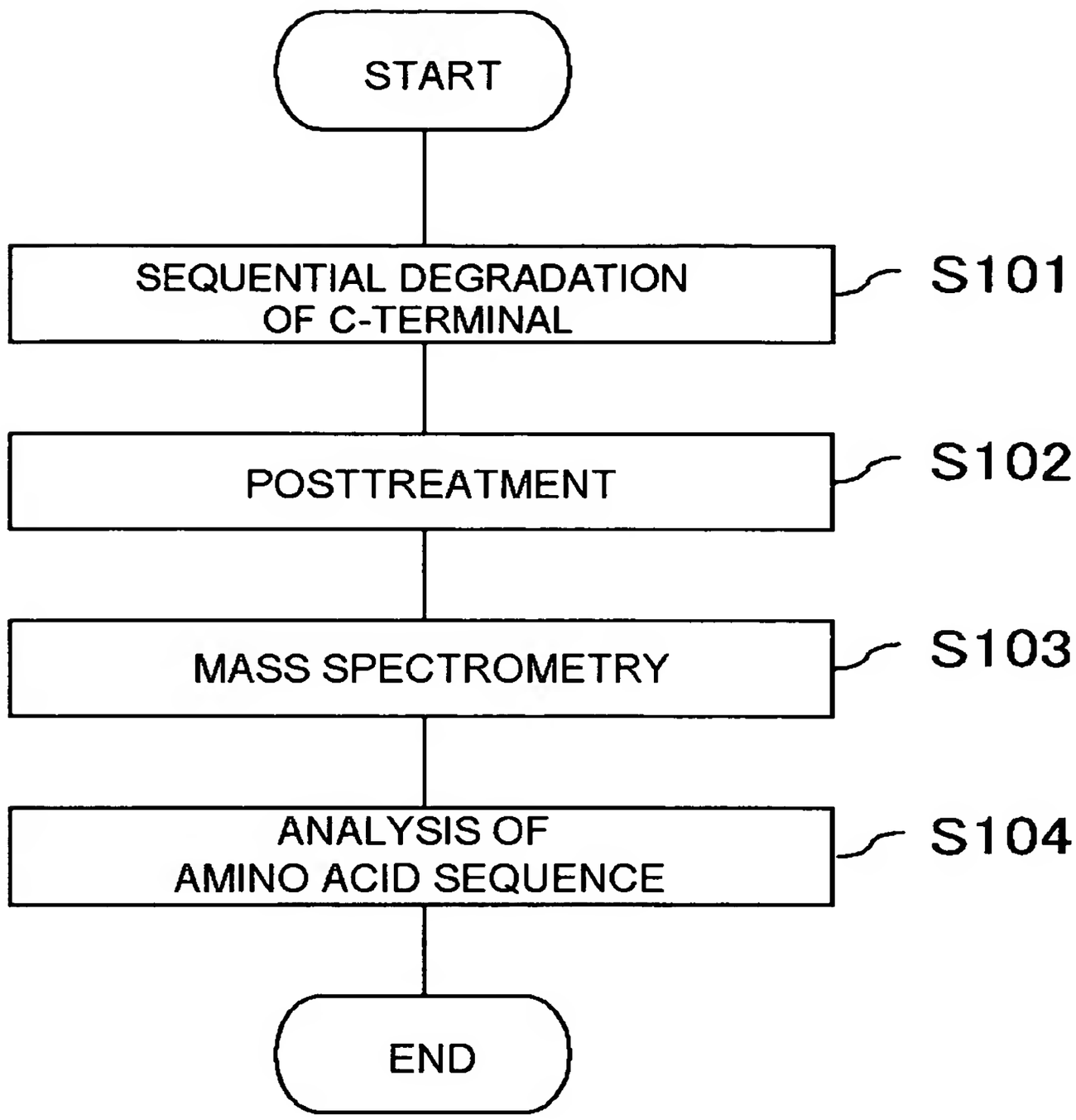

START
SEQUENTIAL DEGRADATION OF C-TERMINAL
S101
POSTTREATMENT
S102
MASS SPECTROMETRY
S103
ANALYSIS OF AMINO ACID SEQUENCE
S104
END

FIG. 2

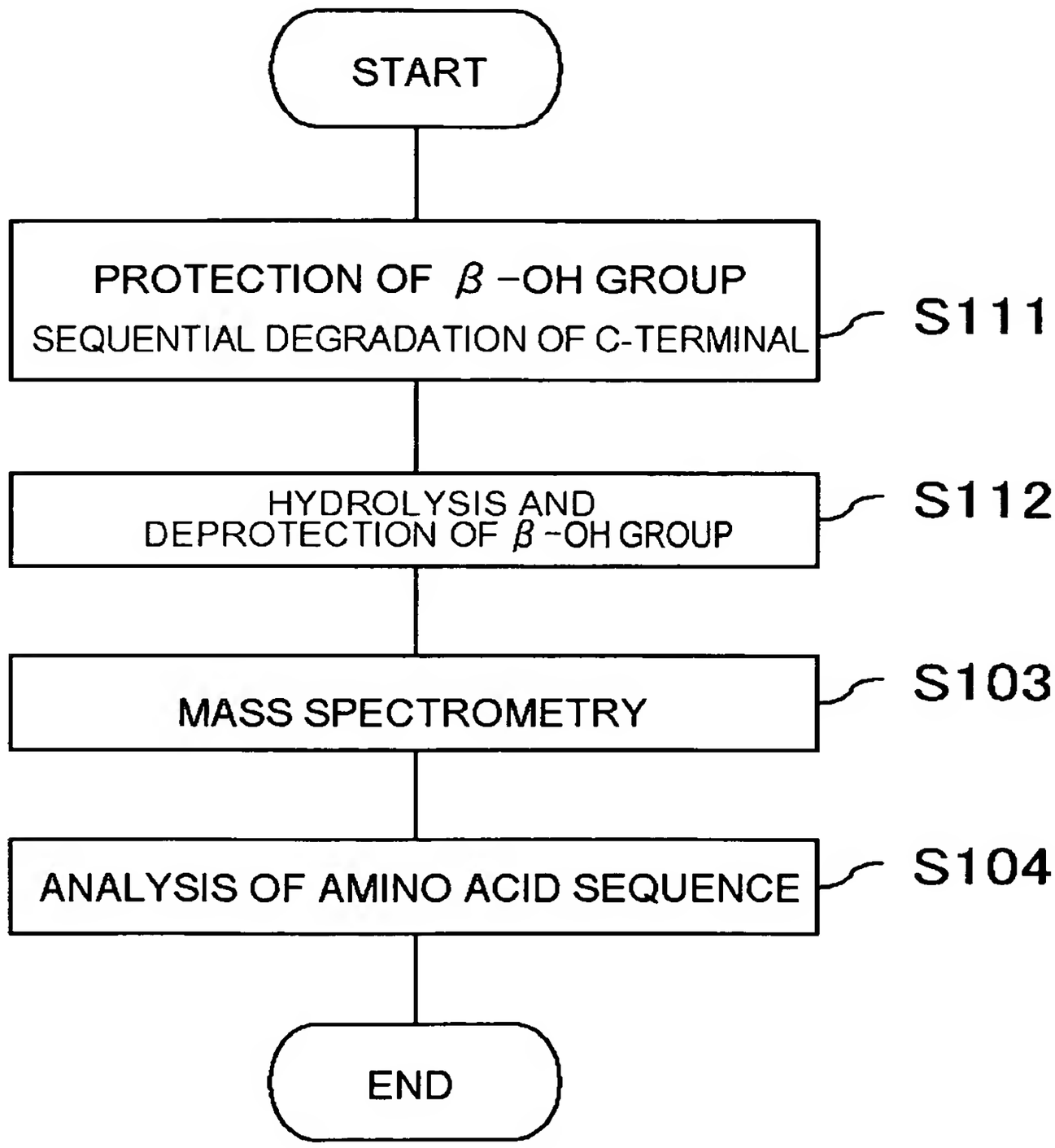

START
PROTECTION OF β -OH GROUP
SEQUENTIAL DEGRADATION OF C-TERMINAL
S111
HYDROLYSIS AND
DEPROTECTION OF β -OH GROUP
S112
MASS SPECTROMETRY
S103
ANALYSIS OF AMINO ACID SEQUENCE
S104
END

FIG. 3

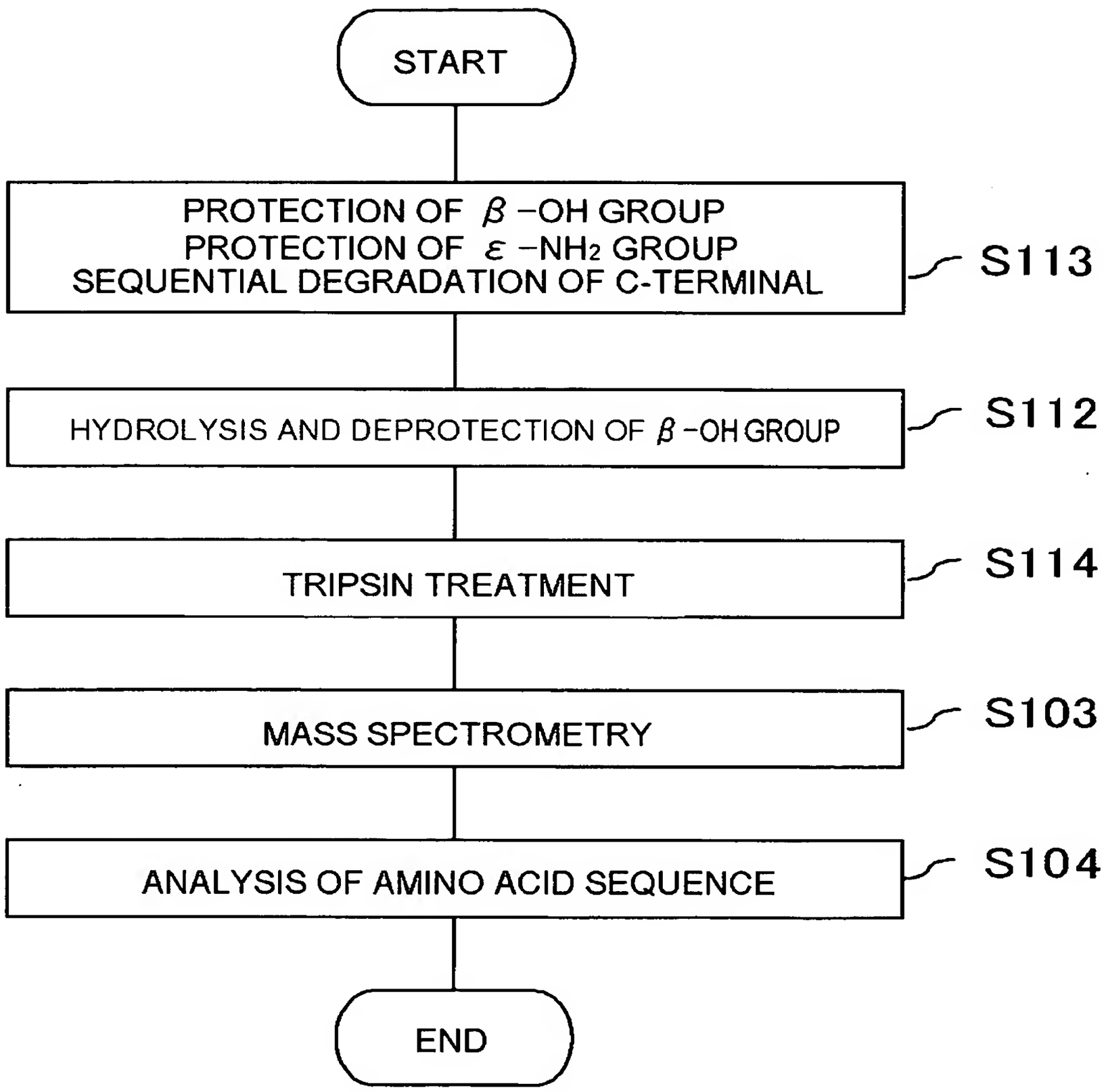

START
PROTECTION OF β -OH GROUP
PROTECTION OF ε -NH2 GROUP
SEQUENTIAL DEGRADATION OF C-TERMINAL
S113
HYDROLYSIS AND DEPROTECTION OF β -OH GROUP
S112
TRIPSIN TREATMENT
S114
MASS SPECTROMETRY
S103
ANALYSIS OF AMINO ACID SEQUENCE
S104
END

FIG. 4

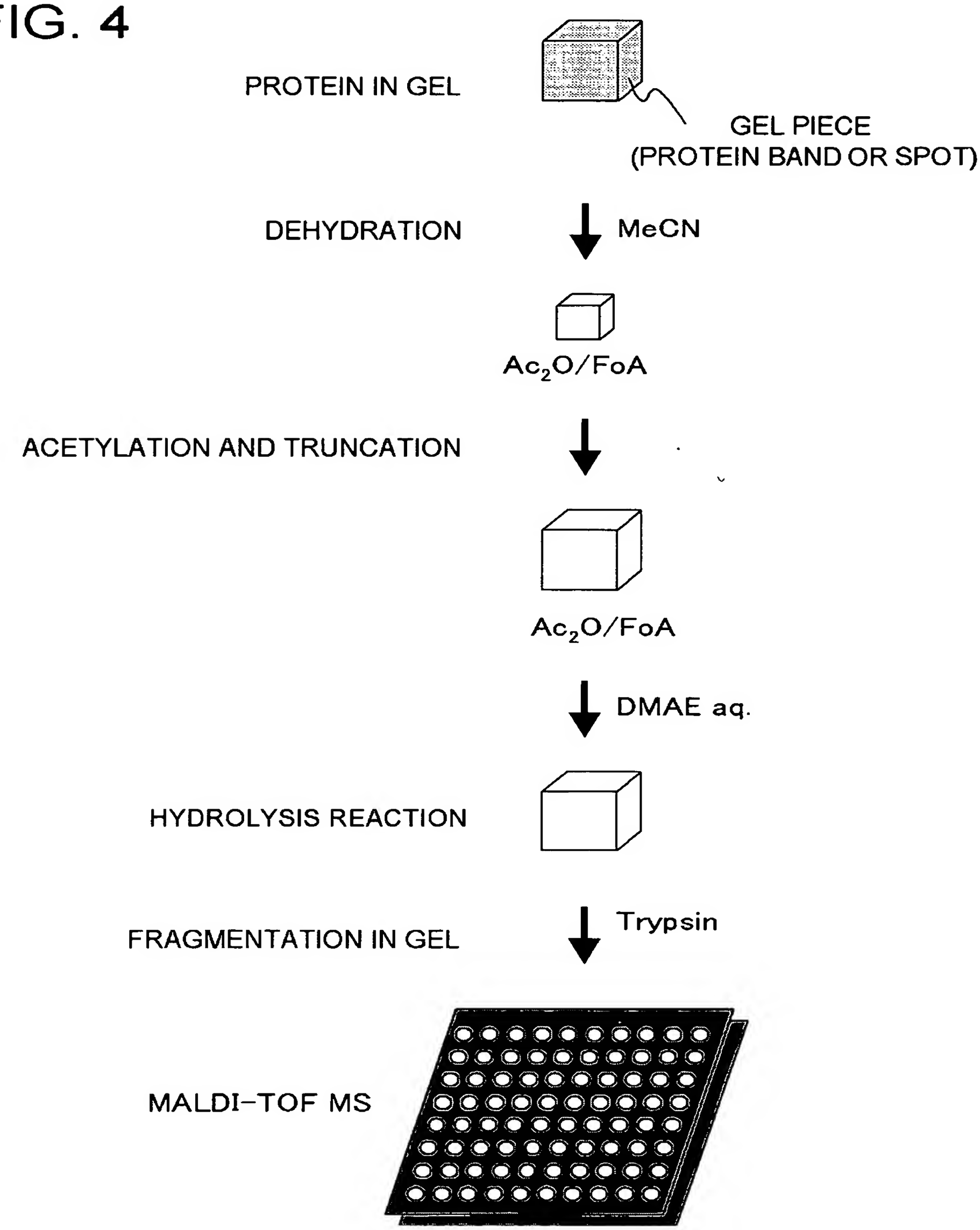

PROTEIN IN GEL
GEL PIECE
(PROTEIN BAND OR SPOT)
DEHYDRATION
MeCN
Ac2O/FoA
ACETYLATION AND TRUNCATION
Ac2O/FoA
DMAE aq.
HYDROLYSIS REACTION
FRAGMENTATION IN GEL
Trypsin
MALDI-TOF MS

FIG. 5

REACTION CONDITION OF ACETYLATION AND TRUNCATION OF PROTEIN IN GEL
(WITHOUT USE OF PERFLUORIC ACID)

REACTION	REAGENT COMPOSITION	TEMPERATURE	PERIOD
ACETYLATION AND TRUNCATION	1%～30% Ac_2O/FoA	50～100℃	4 TO 110 HOURS

REACTION CONDITION OF HYDROLYSIS OF PROTEIN IN GEL

REACTION	REAGENT COMPOSITION	TEMPERATURE	PERIOD
HYDROLYSIS	10～20% AQUEOUS DMAE SOLUTION	50～70℃	30 TO 120 MINUTES

FIG. 6

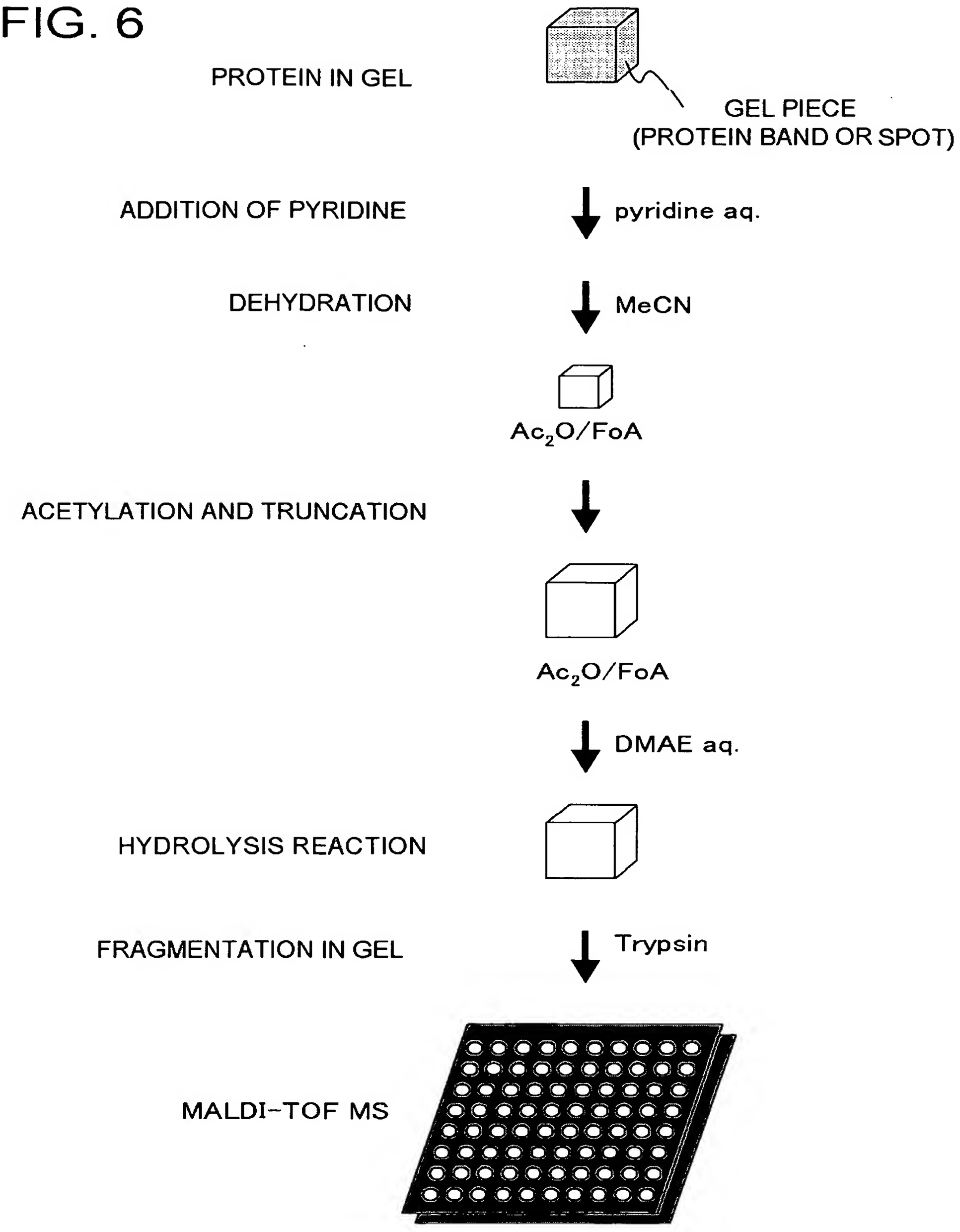

PROTEIN IN GEL
GEL PIECE
(PROTEIN BAND OR SPOT)
ADDITION OF PYRIDINE
pyridine aq.
DEHYDRATION
MeCN
Ac2O/FoA
ACETYLATION AND TRUNCATION
Ac2O/FoA
DMAE aq.
HYDROLYSIS REACTION
FRAGMENTATION IN GEL
Trypsin
MALDI-TOF MS

FIG. 7

myoglobin-horse

[1-153] mass = 17738.180
Cleavage at R

Small polar:	D(7)	E(13)	N(3)	Q(6)			
Large polar:	K(19)	R(2)	H(11)				
Small non-polar:	S(5)	T(7)	A(15)	G(15)			
Large non-polar:	L(17)	I(9)	V(7)	M(2)	F(7)	Y(2)	W(2)
Special:	C(0)	P(4)					

K[16] + 42.04	K[42] + 42.04	K[45] + 42.04	K[47] + 42.04
K[50] + 42.04	K[56] + 42.04	K[62] + 42.04	K[63] + 42.04
K[77] + 42.04	K[78] + 42.04	K[79] + 42.04	K[87] + 42.04
K[96] + 42.04	K[98] + 42.04	K[102] + 42.04	K[118] + 42.04
K[133] + 42.04	K[145] + 42.04	K[147] + 42.04	

```
  1  G L S D G E W Q Q V L N V W G K V E A D I A G H G Q E V L I  30
 31  R I f t g h p e t l e k f d k f k h l k t e a e m k a s e d  60
 61  l k k h g t v v l t a l g g i l k k k g h h e a e l k p l a  90
 91  q s h a t k h k i p i k y l e f i s d a i i h v l h s k h p  120
121  g n f g a d a q g a m t k a l e l f r N D I A A K Y K E L G  150
151  F Q G                                                     153
```

(1) [1-31] = 3444.742 (2) [32-139] = 12692.649 (3) [140-153] = 1636.809

FIG. 8

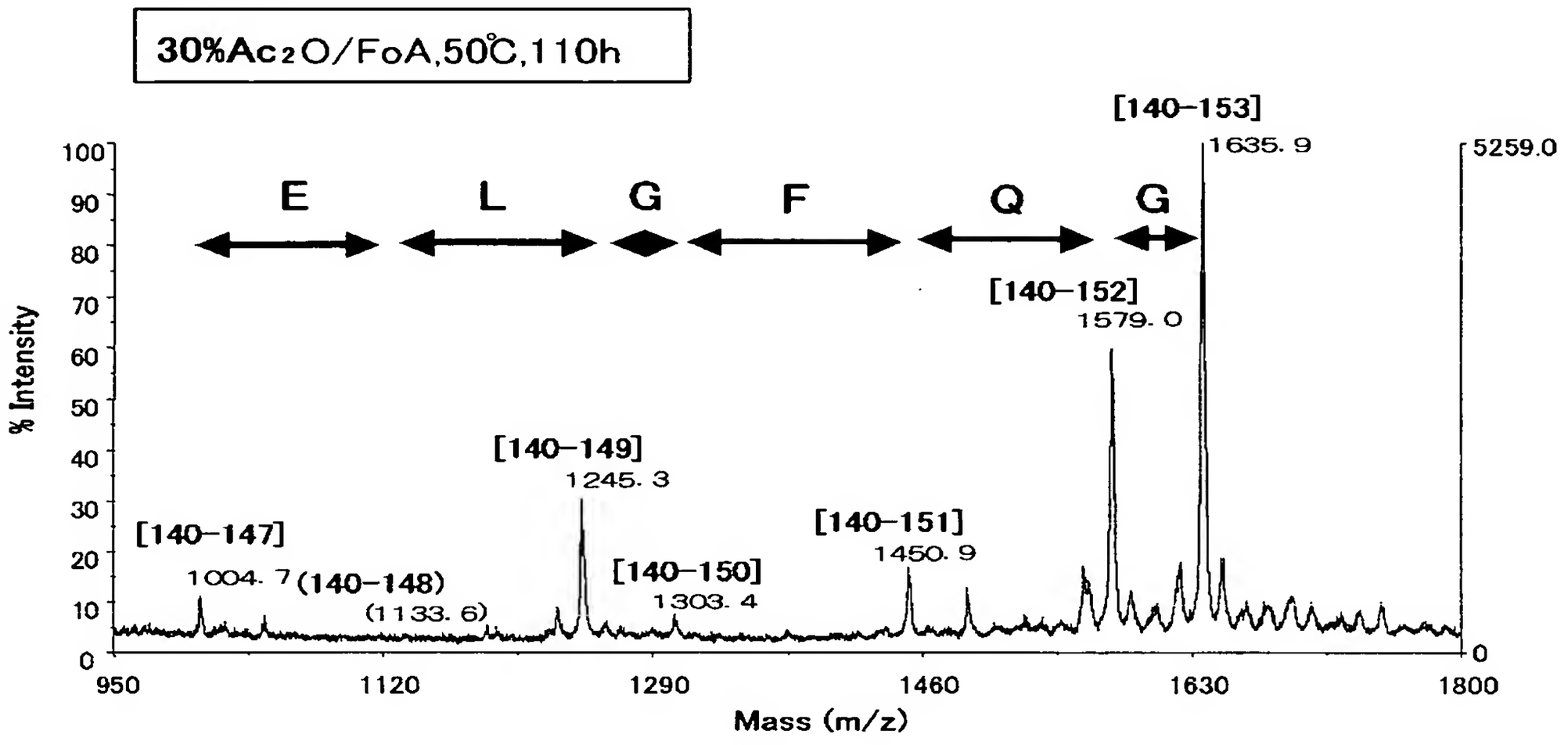

30%Ac2O/FoA,50℃,110h
[140–153]
1635.9
5259.0
E
L
G
F
Q
G
[140–152]
1579.0
[140–149]
1245.3
[140–147]
1004.7
[140–148]
(1133.6)
[140–150]
1303.4
[140–151]
1450.9
% Intensity
100
90
80
70
60
50
40
30
20
10
0
950
1120
1290
1460
1630
1800
Mass (m/z)

FIG. 9

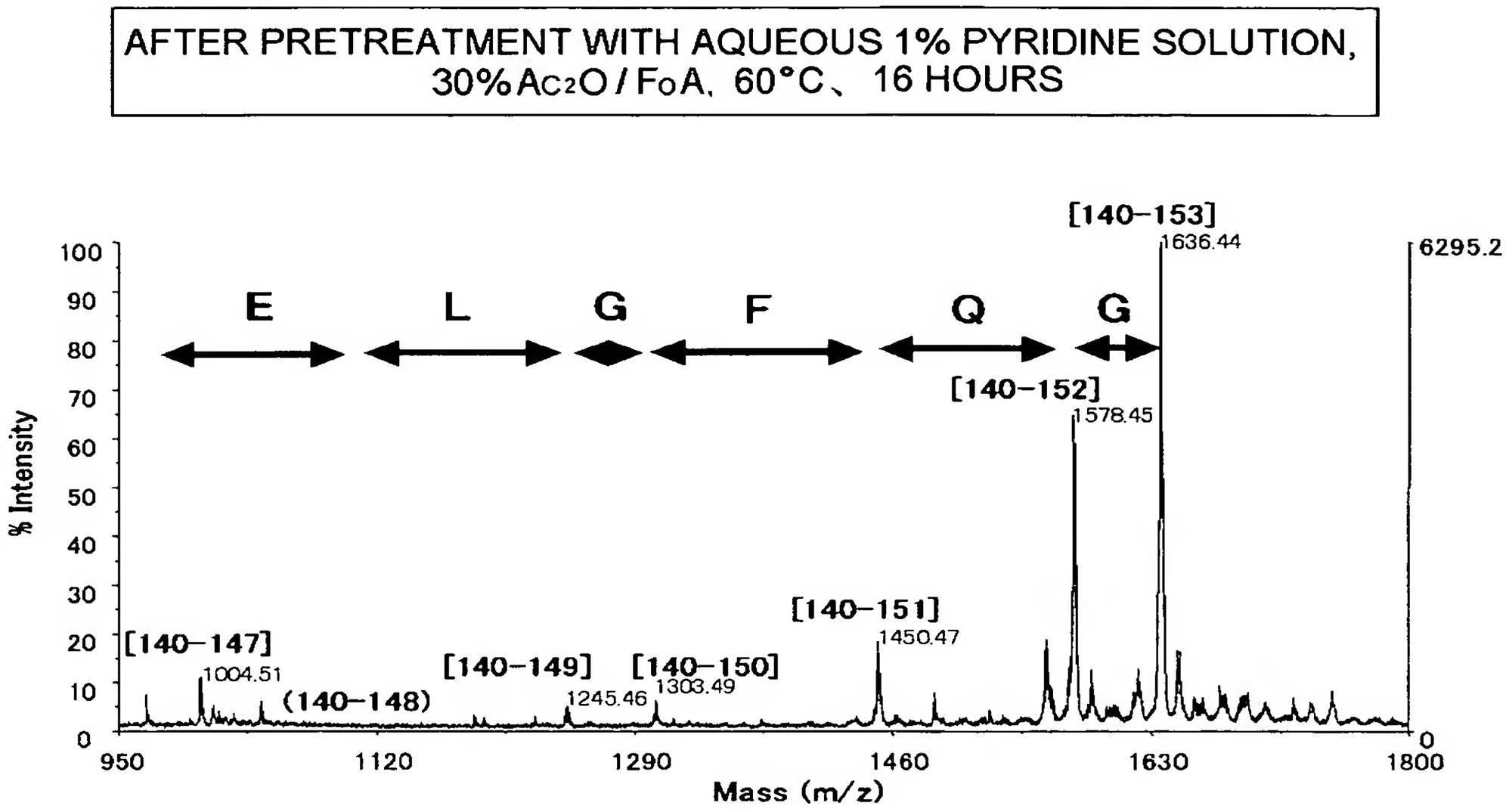

AFTER PRETREATMENT WITH AQUEOUS 1% PYRIDINE SOLUTION,
30% Ac2O / FoA, 60°C、 16 HOURS
[140–153]
E
L
G
F
Q
G
[140–152]
1636.44
6295.2
1578.45
[140–151]
1450.47
[140–147]
1004.51
(140–148)
[140–149]
1245.46
[140–150]
1303.49
% Intensity
100
90
80
70
60
50
40
30
20
10
0
950
1120
1290
1460
1630
1800
Mass (m/z)

FIG. 10

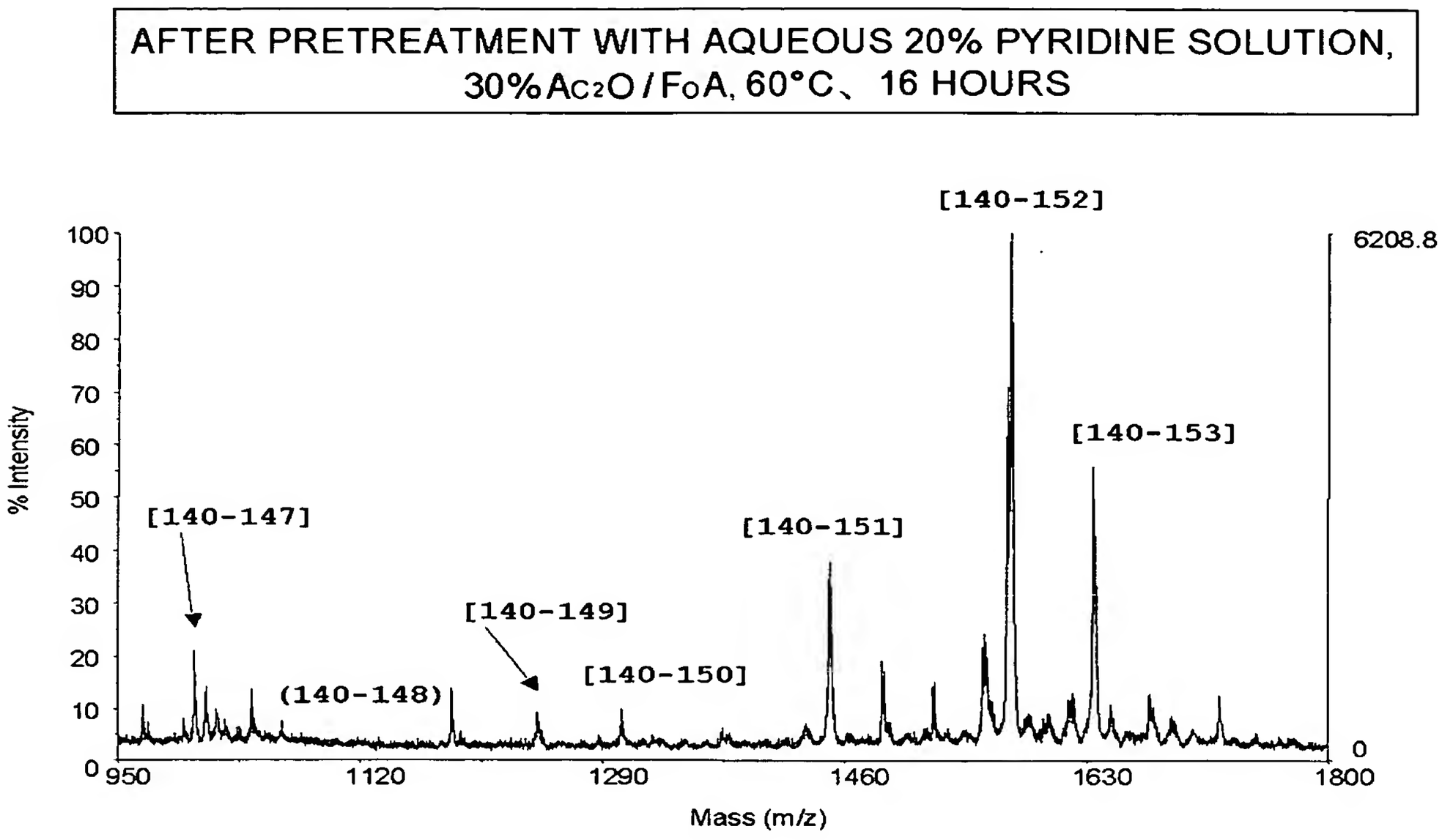

AFTER PRETREATMENT WITH AQUEOUS 20% PYRIDINE SOLUTION,
30% Ac2O / FoA, 60°C、 16 HOURS
[140-152]
6208.8
100
90
80
70
60
50
40
30
20
10
0
% Intensity
[140-147]
[140-151]
[140-153]
[140-149]
[140-150]
(140-148)
0
950
1120
1290
1460
1630
1800
Mass (m/z)

FIG. 11

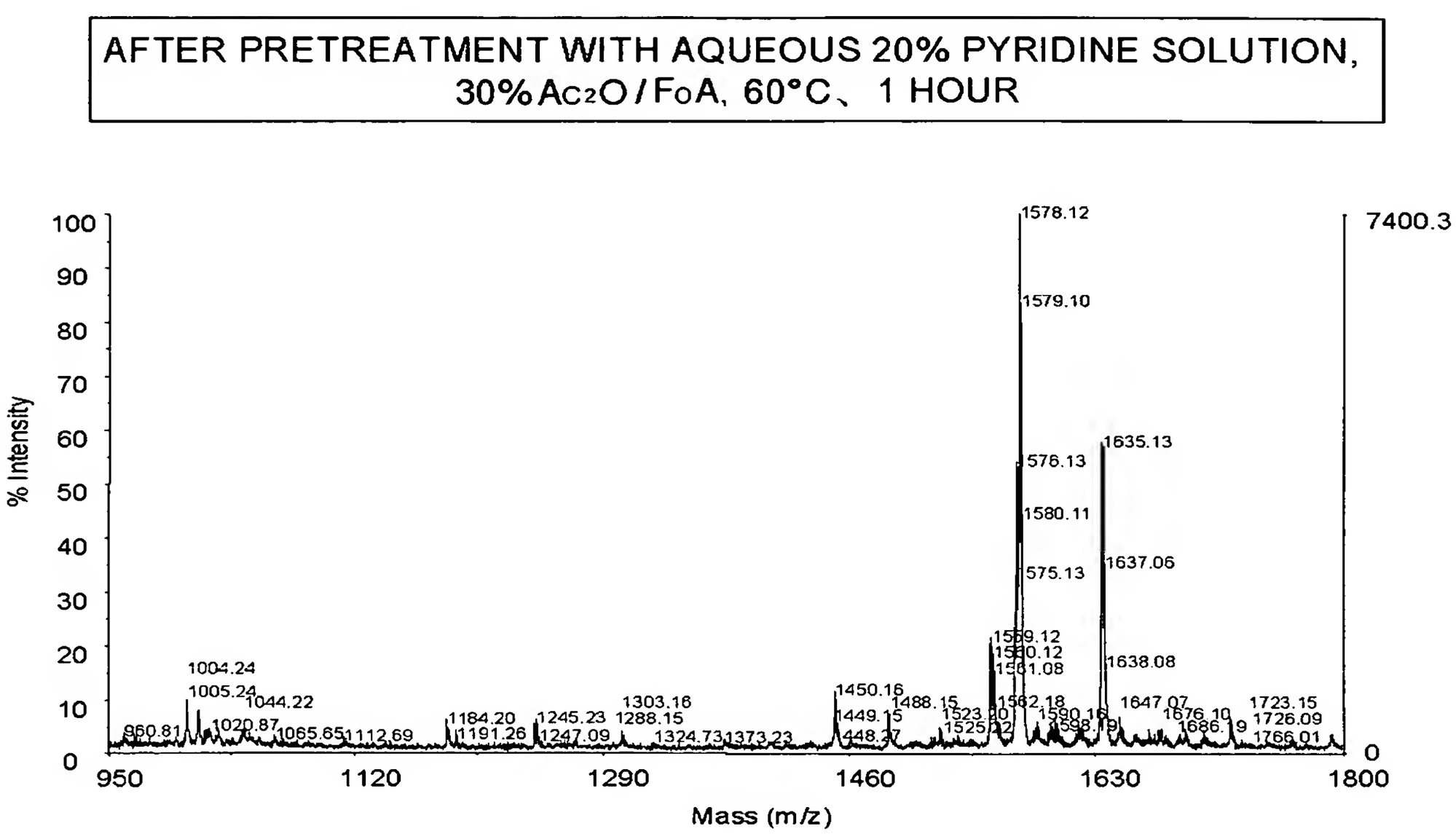

AFTER PRETREATMENT WITH AQUEOUS 20% PYRIDINE SOLUTION,
30% Ac2O / FoA, 60°C、 1 HOUR
% Intensity
Mass (m/z)
7400.3
1578.12
1579.10
1635.13
1576.13
1580.11
1637.06
1575.13
1638.08
1004.24
1005.24
1044.22
1020.87
960.81
1065.65
1112.69
1184.20
1191.26
1245.23
1247.09
1303.16
1288.15
1324.73
1373.23
1450.16
1449.15
1448.27
1488.15
1647.07
1723.15
1726.09
1766.01

FIG. 12

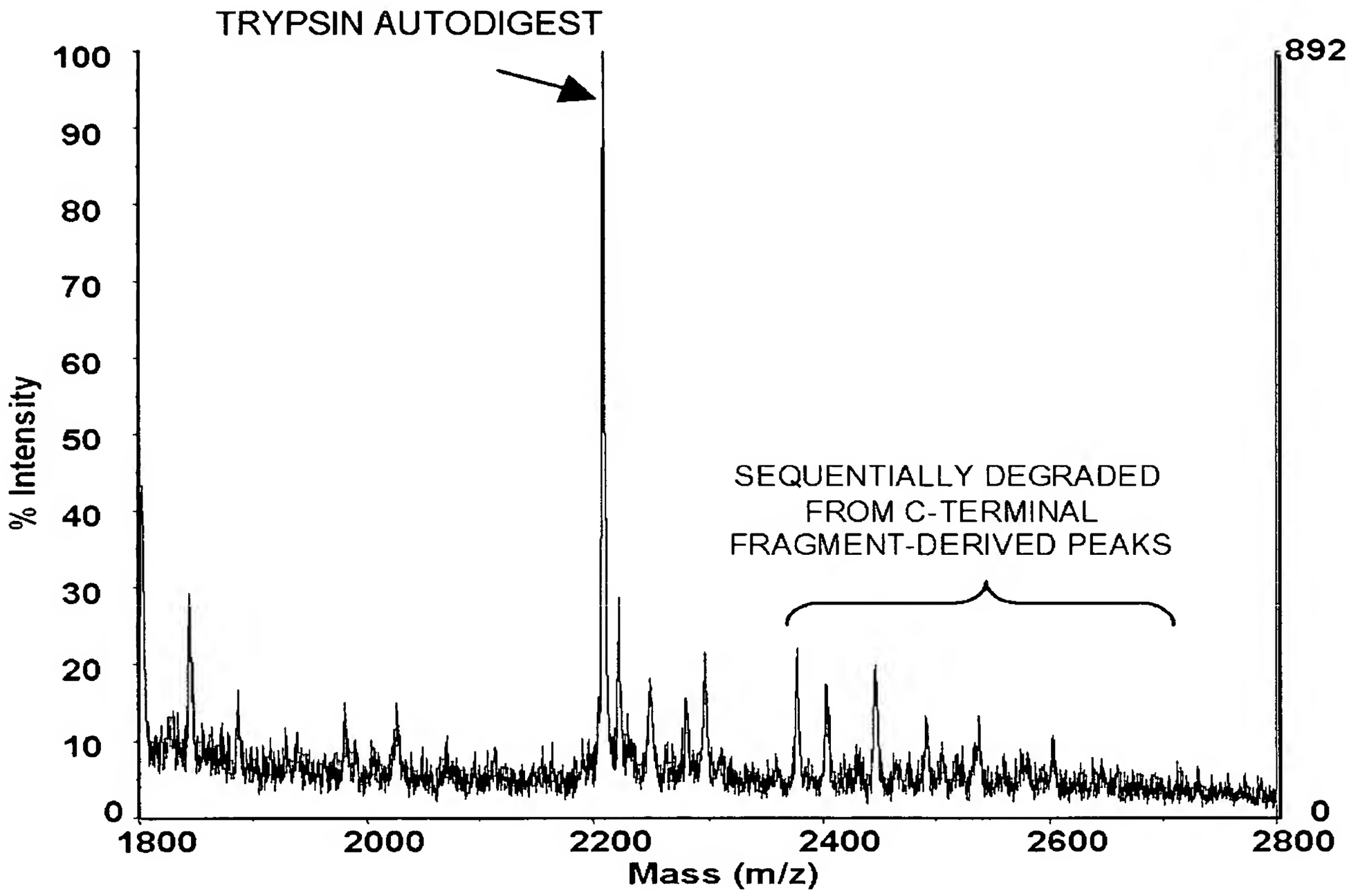
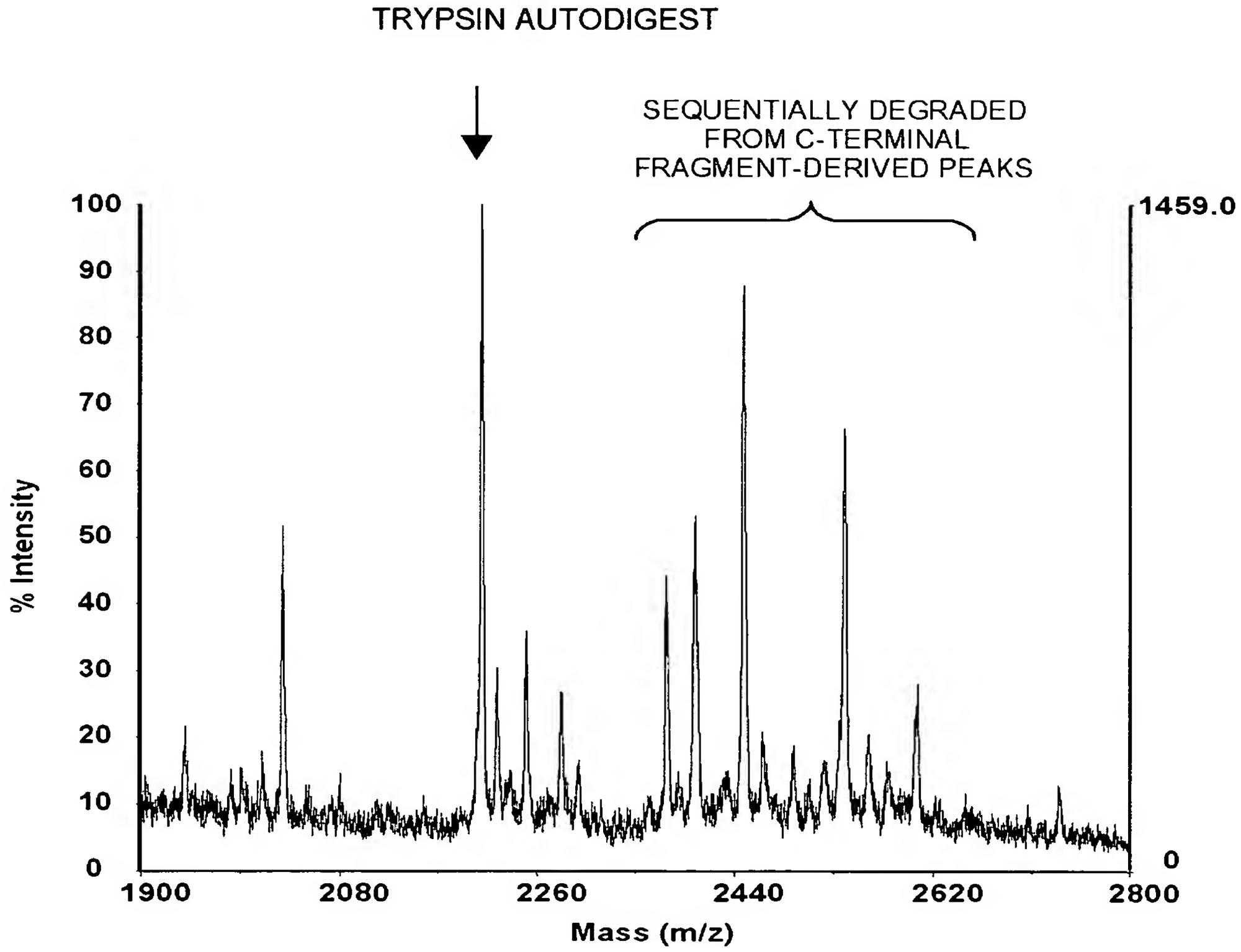

TRYPSIN AUTODIGEST
892.3
SEQUENTIALLY DEGRADED
FROM C-TERMINAL
FRAGMENT-DERIVED PEAKS
% Intensity
100
90
80
70
60
50
40
30
20
10
0
1800
2000
2200
2400
2600
2800
Mass (m/z)

FIG. 13


TRYPSIN AUTODIGEST
SEQUENTIALLY DEGRADED
FROM C-TERMINAL
FRAGMENT-DERIVED PEAKS
1459.0
100
90
80
70
60
50
40
30
20
10
0
% Intensity
0
1900
2080
2260
2440
2620
2800
Mass (m/z)